

ANNEX II

REQUIREMENTS FOR THE DOSSIER TO BE SUBMITTED FOR
THE INCLUSION OF AN ACTIVE SUBSTANCE IN ANNEX I

PART A

Chemical substances⁽¹⁾**[F18. Ecotoxicological studies****Introduction**

- (i) The information provided, taken together with that for one or more preparations containing the active substance, must be sufficient to permit an assessment of the impact on non-target species (flora and fauna), likely to be at risk from exposure to the active substance, its metabolites, degradation and reaction products, where they are of environmental significance. Impact can result from single, prolonged or repeated exposure and can be reversible or irreversible.
- (ii) In particular, the information provided for the active substance, together with other relevant information, and that provided for one or more preparations containing it, should be sufficient to:
 - decide whether, or not, the active substance can be included in Annex I,
 - specify appropriate conditions or restrictions to be associated with any inclusion in Annex I,
 - permit an evaluation of short- and long-term risks for non-target species — populations, communities, and processes — as appropriate,
 - classify the active substance as to hazard,
 - specify the precautions necessary for the protection of non-target species, and
 - specify the hazard symbols, the indications of danger, and relevant risk and safety phrases for the protection of the environment, to be mentioned on packaging (containers).
- (iii) There is a need to report all potentially adverse effects found during routine ecotoxicological investigations and to undertake and report, where required by the competent authorities, such additional studies which may be necessary to investigate the probable mechanisms involved and assess the significance of these effects. All available biological data and information which is relevant to the assessment of the ecotoxicological profile of the active substance must be reported.
- (iv) The information on fate and behaviour in the environment, generated and submitted in accordance with points 7.1 to 7.4, and on residue levels in plants generated and submitted in accordance with point 6 is central to the assessment of impact on non-target species, in that together with information on the nature of the preparation and its manner of use, it defines the nature and extent of potential exposure. The toxicokinetic and toxicological studies and information submitted in accordance with points 5.1 to 5.8 provide essential information as to toxicity to vertebrate species and the mechanisms involved.
- (v) Where relevant, tests should be designed and data analysed using appropriate statistical methods. Full details of the statistical analysis should be reported (e. g. all

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point estimates should be given with confidence intervals, exact p-values should be given rather than stating significant/non significant).

Test substance

- (vi) A detailed description (specification) of the material used, as provided for under point 1.11 must be provided. Where testing is done using active substance the material used should be of that specification that will be used in the manufacture of preparations to be authorized except where radiolabelled material is used.
- (vii) Where studies are conducted using active substance produced in the laboratory or in a pilot plant production system, the studies must be repeated using active substance as manufactured, unless it can be justified that the test material used is essentially the same, for the purposes of ecotoxicological testing and assessment. In cases of uncertainty, appropriate bridging studies must be submitted to serve as a basis for a decision as to the possible need for repetition of the studies.
- (viii) In the case of studies in which dosing extends over a period, dosing should preferably be done using a single batch of active substance if stability permits.

Whenever a study implies the use of different doses, the relationship between dose and adverse effect must be reported.
- (ix) For all feeding studies, average achieved dose must be reported, including where possible the dose in mg/kg body weight. Where dosing via the diet is utilized the test compound must be distributed uniformly in the diet.
- (x) It may be necessary to conduct separate studies for metabolites, degradation or reaction products, where these products can constitute a relevant risk to non-target organisms and where their effects cannot be evaluated by the available results relating to the active substance. Before such studies are performed the information from points 5, 6 and 7 has to be taken into account.

Test organisms

- (xi) In order to facilitate the assessment of the significance of test results obtained, including the estimation of intrinsic toxicity and the factors affecting toxicity, the same strain (or recorded origin) of each relevant species should, where possible, be used in the various toxicity tests specified.

8.1. Effects on birds

8.1.1. Acute oral toxicity

Aim of the test

The test should provide, where possible, LD₅₀ values, the lethal threshold dose, time courses of response and recovery and the NOEL, and must include relevant gross pathological findings. Circumstances in which required

The possible effects of the active substance on birds must be investigated except where the active substance is intended solely to be included in preparations for exclusive use in enclosed spaces (e.g. in glasshouses or in food storage practice).

Test conditions

The acute oral toxicity of active substance to a quail species (Japanese quail (*Coturnix coturnix japonica*) or Bobwhite quail (*Colinus virginianus*) or to mallard duck (*Anas platyrhynchos*) must be determined. The highest dose used in tests need not exceed 2 000 mg/kg body weight.

Test guideline

Setac — Procedures for assessing the environmental fate and ecotoxicity of pesticides⁽²⁾.

8.1.2. Short-term dietary toxicity

Aim of the test

The test should provide the short term dietary toxicity (LC₅₀ values, lowest lethal concentration (LLC), where possible no observed effect concentrations (NOEC), time courses of response and recovery) and include relevant gross pathological findings.

Circumstances in which required

The dietary (five-day) toxicity of the active substance to birds must always be investigated on one species except where a study in accordance with the provisions of point 8.1.3 is reported. Where its acute oral NOEL is ≤ 500 mg/kg body weight or where the short-term NOEC < 500 mg/kg food the test must be performed on a second species.

Test conditions

The first species to be studied must be either a quail species or mallard duck. If a second species must be tested it should not be related to the first species tested.

Test guideline

The test must be carried out in accordance with OECD Method 205.

8.1.3. Subchronic toxicity and reproduction

Aim of the test

The test should provide the subchronic toxicity and reproductive toxicity of the active substance to birds.

Circumstances in which required

The subchronic and reproductive toxicity of the active substance to birds must be investigated, unless it can be justified that continued or repeated exposure of adults, or exposure of nest sites during the breeding season is unlikely to occur.

Test guideline

The test must be carried out in accordance with OECD Method 206.

8.2. Effects on aquatic organisms

The data of the tests referred to in points 8.2.1, 8.2.4 and 8.2.6 have to be submitted for every active substance even when it is not expected that plant protection products containing it could reach surface water following the proposed conditions of use. These data are required under the provisions of Annex VI to Directive 67/548/EEC for the classification of the active substance.

Data reported must be supported with analytical data on concentrations of the test substance in the test media.

8.2.1. Acute toxicity to fish

Aim of the test

The test should provide the acute toxicity (LC₅₀), and details of observed effects.

Circumstances in which required

The test must always be carried out.

Test conditions

The acute toxicity of the active substance must be determined for rainbow trout (*Oncorhynchus mykiss*) and for a warm water fish species. Where tests with metabolites, degradation or reaction

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products have to be performed the species used must be the more sensitive of the two species tested with the active substance.

Test guideline

The test must be carried out in accordance with the Annex to Commission Directive 92/69/EEC⁽³⁾ adapting to technical progress for the 17th time Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification and labelling of dangerous substances, Method C1.

8.2.2. Chronic toxicity to fish

Circumstances in which required

A chronic toxicity study must be carried out unless it can be justified that continued or repeated exposure of fish is unlikely to occur or unless a suitable microsom or mesocosm study is available.

Expert judgment is required to decide which test has to be performed. In particular for active substance for which there are indications of particular concerns (related to the toxicity of the active substance for fish or the potential exposure) the applicant shall seek the agreement of the competent authorities on the type of test to be performed.

A fish early life stage toxicity test might be appropriate where bioconcentration factors (BCF) are between 100 and 1 000 or where EC_{50} of the active substance $< 0,1$ mg/l.

A fish life cycle test might be appropriate in cases where

- the bioconcentration factor is greater than 1 000 and the elimination of the active substance during a depuration phase of 14 days is lower than 95 %,
- or
- the substance is stable in water or sediment ($DT_{90} > 100$ days).

It is not necessary to perform a chronic toxicity test on juvenile fish when a fish early life stage toxicity test or a fish life cycle test has been performed; it is likewise not necessary to perform a fish early life stage toxicity test when a fish life cycle test has been performed.

8.2.2.1. Chronic toxicity test on juvenile fish

Aim of the test

The test should provide effects on growth, the threshold level for lethal effects and for observed effects, the NOEC and details of observed effects.

Test conditions

The test must be conducted on juvenile rainbow trout, following exposure of 28 days to the active substance. Data on the effects on growth and behaviour must be generated.

8.2.2.2. Fish early life stage toxicity test

Aim of the test

The test should provide effects on development, growth and behaviour, the NOEC and details of observed effects on fish early life stages.

Test guideline

The test must be carried out in accordance with OECD Method 210.

8.2.2.3. Fish life cycle test

Aim of the test

The test will provide effects on reproduction of the parental and the viability of the filial generation.

Test conditions

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.2.3. Bioconcentration in fish

Aim of the test

The test should provide the steady-state bioconcentration factors, uptake rate constants and depuration rate constants, calculated for each test compound, as well as relevant confidence limits.

Circumstances in which required

The bioconcentration potential of active substances, of metabolites and of degradation and reaction products, likely to partition into fatty tissues (such as $\log p_{ow} \geq 3$ — see point 2.8 or other relevant indications of bioconcentration), must be investigated and be reported, unless it can be justified that exposure leading to bioconcentration is not likely to occur.

Test guideline

The test must be carried out in accordance with OECD Method 305E.

8.2.4. Acute toxicity to aquatic invertebrates

Aim of the test

The test should provide the 24 and 48-hour acute toxicity of the active substance, expressed as the median effective concentration (EC_{50}) for immobilization, and where possible the highest concentration causing no immobilization.

Circumstances in which required

The acute toxicity must always be determined for *Daphnia* (preferably *Daphnia magna*). Where plant protection products containing the active substance are intended to be used directly on surface water additional data have to be reported on at least one representative species from each of the following groups: aquatic insects, aquatic crustaceans (on a species not related to *Daphnia*) and aquatic gastropod molluscs.

Test guideline

The test must be carried out in accordance with Directive 92/69/EEC, Method C2.

8.2.5. Chronic toxicity to aquatic invertebrates

Aim of the test

The test should provide where possible EC_{50} values for effects such as immobilization and reproduction and the highest concentration at which no effect such as on mortality or reproduction occurs (NOEC) and details of observed effects.

Circumstances in which required

A test on *Daphnia* and on at least one representative aquatic insect species and an aquatic gastropod mollusc species must be carried out unless it can be justified that continued or repeated exposure is not likely to occur.

Test conditions

The test with *Daphnia* must be continued for 21 days.

Test guideline

The test must be carried out in accordance with OECD Method 202, Part II.

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8.2.6. Effects on algal growth

Aim of the test

The test should provide EC₅₀ values for growth and growth rate, NOEC values, and details of observed effects.

Circumstances in which required

Possible effects on algal growth of active substances must always be reported.

For herbicides a test on a second species from a different taxonomic group has to be performed.

Test guideline

The test must be carried out in accordance with Directive 92/69/EEC, Method C3.

8.2.7. Effects on sediment dwelling organisms

Aim of test

The test will measure effects on survival and development (including effects on emergence of adults for *Chironomus*), the relevant EC₅₀ values and the NOEC values.

Circumstances in which required

Where environmental fate and behaviour data required in point 7 report that an active substance is likely to partition to and persist in aquatic sediments, expert judgement should be used to decide whether an acute or a chronic sediment toxicity test is required. Such expert judgement should take into account whether effects on sediment dwelling invertebrates are likely by comparing the aquatic invertebrate toxicity EC₅₀ data from points 8.2.4 and 8.2.5 with the predicted levels of the active substances in sediment from data in Annex III, point 9.

Test conditions

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.2.8. Aquatic plants

A test on aquatic plants has to be performed for herbicides.

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.3. Effect on arthropods

8.3.1. Bees

8.3.1.1. Acute toxicity

Aim of the test

The test should provide the acute oral and contact LD₅₀ value of the active substance.

Circumstances in which required

Potential impact on bees must be investigated, except where preparations containing the active substance are for exclusive use in situations where bees are not likely to be exposed such as:

- food storage in enclosed spaces,
- non-systemic seed dressings,
- non-systemic preparations for application to soil,
- non-systemic dipping treatments for transplanted crops and bulbs,
- wound sealing and healing treatments,

- rodenticidal baits,
- use in glasshouses without pollinators.

Test guideline

The test must be carried out in accordance with EPPO Guideline 170.

8.3.1.2. Bee brood feeding test

Aim of the test

The test should provide sufficient information to evaluate possible risks from the plant protection product on honeybee larvae.

Circumstances in which required

The test must be carried out when the active substance may act as an insect growth regulator unless it can be justified that it is not likely that bee brood would be exposed to it.

Test guideline

The test must be carried out in accordance with ICPBR Method (e.g. P. A. Oomen, A. de Riufter and J. van der Steen. Method for honeybee brood feeding tests with insect growth-regulating insecticides. *EPPO Bulletin*, Volume 22, pp 613 to 616, 1992.)

8.3.2. Other arthropods

Aim of the test

The test should provide sufficient information to evaluate the toxicity (mortality and sublethal effects) of the active substance to selected arthropod species.

Circumstances in which required

Effects on non-target terrestrial arthropods (e.g. predators or parasitoids of harmful organisms) must be investigated. The information obtained for these species can also be used to indicate the potential for toxicity to other non-target species inhabiting the same environment. This information is required for all active substances except where preparations containing the active substance are for exclusive use in situations where non-target arthropods are not exposed such as:

- food storage in enclosed spaces,
- wound sealing and healing treatments,
- rodenticidal baits.

Test conditions

The test must be performed initially in the laboratory on an artificial substrate (i.e. glass plate or quartz sand, as appropriate) unless adverse effects can be clearly predicted from other studies. In these cases, more realistic substrates may be used.

Two sensitive standard species, a parasitoid and predatory mite (e.g. *Aphidius rhopalosiphi* and *Typhlodromus pyri*) should be tested. In addition to these, two additional species must also be tested, which should be relevant to the intended use of the substance. Where possible and if appropriate, they should represent the other two major functional groups, ground dwelling predators and foliage dwelling predators. Where effects are observed with species relevant to the proposed use of the product, further testing may be carried out at the extended laboratory/semi-field level. Selection of the relevant test species should follow the proposals outlined in Setac — Guidance document on regulatory testing procedures for pesticides with non-target arthropods⁽⁴⁾. Testing must be conducted at rates equivalent to the highest rate of field application to be recommended.

Test guideline

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Where relevant, testing should be done according to appropriate guidelines which satisfy at least the requirements for testing as included in Setac — Guidance document on regulatory testing procedures for pesticides with non-target arthropods.

8.4. Effects on earthworms

8.4.1. Acute toxicity

Aim of the test

The test should provide the LC₅₀ value of the active substance to earthworms, where possible the highest concentration causing no mortality and the lowest concentration causing 100 % mortality, and must include observed morphological and behavioural effects.

Circumstances in which required

Effects on earthworms must be investigated, where preparations containing the active substance are applied to soil, or can contaminate soil.

Test guideline

The test must be carried out in accordance with Commission Directive 88/302/EEC⁽⁵⁾ adapting to technical progress for the ninth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, Part C, Toxicity for earthworms: Artificial soil test.

8.4.2. Sublethal effects

Aim of the test

The test should provide the NOEC and the effects on growth, reproduction and behaviour.

Circumstances in which required

Where on the basis of the proposed manner of use of preparations containing the active substance or on the basis of its fate and behaviour in soil (DT₉₀ > 100 days), continued or repeated exposure of earthworms to the active substance, or to significant quantities of metabolites, degradation or reaction products, can be anticipated expert judgement is required to decide whether a sublethal test can be useful.

Test conditions

The test must be carried out on *Eisenia foetida*.

8.5. Effects on soil non-target micro-organisms

Aim of the test

The test should provide sufficient data to evaluate the impact of the active substance on soil microbial activity, in terms of nitrogen transformation and carbon mineralization.

Circumstances in which required

The test must be carried out where preparations containing the active substance are applied to soil or can contaminate soil under practical conditions of use. In the case of active substances intended for use in preparations for soil sterilization, the studies must be designed to measure rates of recovery following treatment.

Test conditions

Soils used must be freshly sampled agricultural soils. The sites from which soil is taken must not have been treated during the previous two years with any substance that could substantially alter the diversity and levels of microbial populations present, other than in a transitory manner.

Test guideline

Setac — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

8.6. Effects on other non-target organisms (flora and fauna) believed to be at risk

A summary of available data from preliminary tests used to assess the biological activity and dose range finding, whether positive or negative, which may provide information with respect to possible impact on other non-target species, both flora and fauna, must be provided, together with a critical assessment as to its relevance to potential impact on non-target species.

8.7. Effects on biological methods for sewage treatment

Effects on biological methods for sewage treatment must be reported where the use of plant protection products containing the active substance can give rise to adverse effects on sewage treatment plants.]

Textual Amendments

- F1** Substituted by [Commission Directive 96/12/EC of 8 March 1996 amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market \(Text with EEA relevance\).](#)

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- (1) Substance within the meaning of the definition of Article 2, point 3.
- (2) [^{F1}Society of Environmental Toxicology and Chemistry (Setac), 1995. *Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides*, ISBN 90-5607-002-9.
- (3) [OJ No L 383, 29. 12. 1992, p. 113.](#)
- (4) From the Workshop European Standard Characteristics of beneficials Regulatory Testing (Escort), 28 to 30 March 1994, ISBN 0-95-22535-2-6.
- (5) [OJ No L 133, 30. 5. 1988, p. 1.\]](#)

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